# MAb Charge Variants Analysis (CVA) by pH Gradient Cation Exchange Chromatography - Erbitux on Different Column Vendors

**Mobile phase**:

CX-1 pH Gradient Buffer A, pH 5.6

CX-1 pH Gradient Buffer B, pH 10.2

*Purchased from Thermo Scientific™

**Monoclonal Antibody (mAb) Sample:**

ERBITUX® (cetuximab) 66733-0958-23

# *Charge Variants Analysis of Erbitux® with pH Gradient*
# CEX-HPLC Vendor Comparison

This study discusses the performance comparison of Sepax Proteomix SCX and Proteomix WCX versus the benchmark SCX and WCX chromatography profiles on Erbitux (fusion/chimeric mAb) by using pre-made commercially available pH gradient buffers from Thermo Scientific. Flow rates were normalized for column size so an equal comparison could be made; allowing for simple method development and consistency among both phase types.

1. Proteomix SCX showed better resolution of the basic species versus the benchmark SCX column.
2. Proteomix WCX vs. the benchmark WCX showed better resolution.
3. The Proteomix SCX vs. Proteomix WCX performance comparison is also illustrated in this new application data. Under this specific pH buffer system, for analysis of Erbitux, Proteomix SCX shows better peak separation than Proteomix WCX.
4. The proprietary surface modifications of Sepax's non-porous polymer substrate, as well as the ligand chemistry, results in unique selectivity of ionic variants allowing for greater separation.

It is important to note that Sepax also sells semi-preparative and preparative columns in the same chemistry and particle size, allowing for efficient linear scalability (from ug-->grams) while achieving the same high-resolution separation. Such linear scalability allows for efficient fractionation of charge variants for further characterization.

# Strong Cation Exchange (SCX) Column Comparison

**HPLC Columns**:

Sepax, Proteomix SCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 401NP5P-4625)

Other Vendor SCX PEEK, 5µm, NP 4.0 x 250 mm (P/N: *REDACTED*)

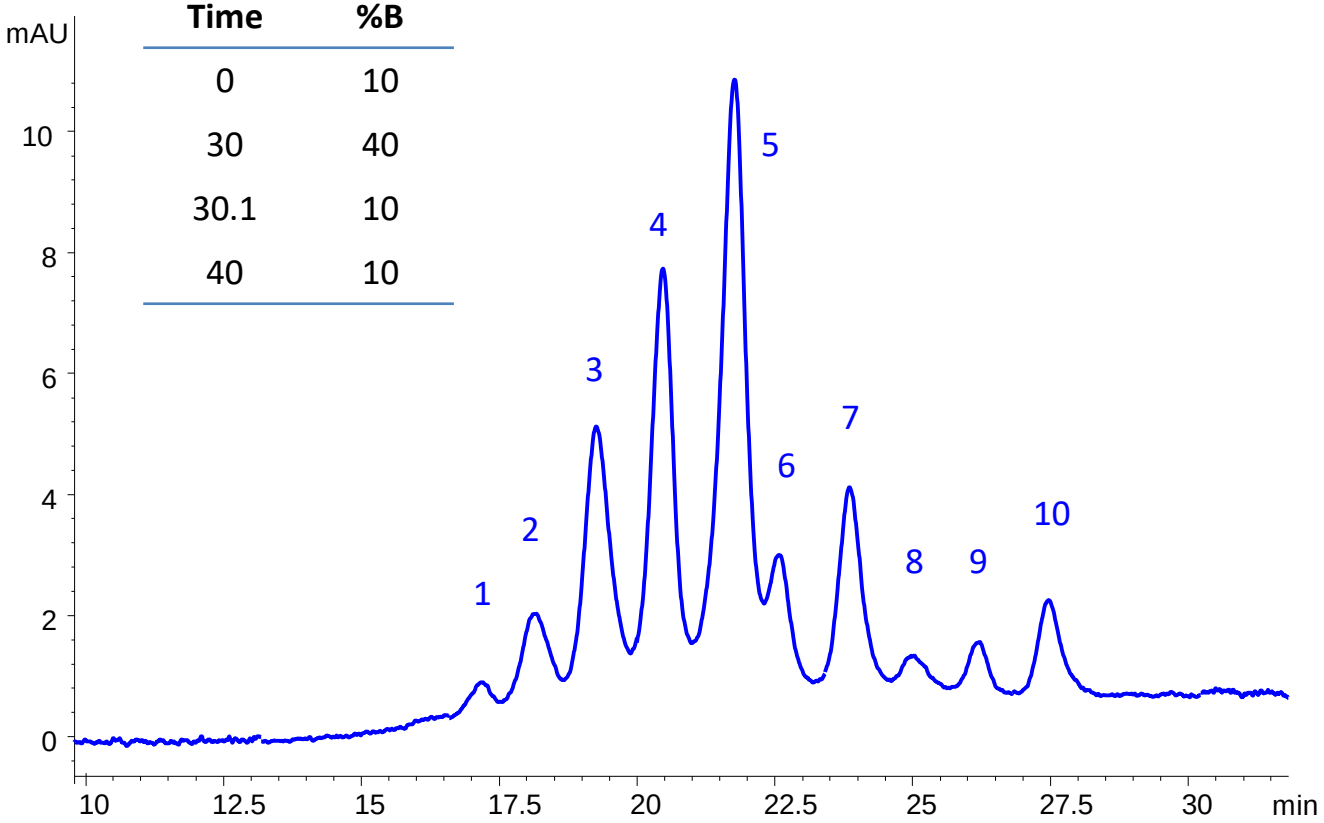
**Flow rates and injection amounts were linearized based on column specifications*

# Erbitux® – *Sepax* Proteomix SCX

Column: Sepax, Proteomix SCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 401NP5P-4625);
Mobile phase: CX-1 pH Buffer A; B: CX-1 pH Buffer B;
Flow velocity: 480 cm/h, Detector: UV 280 nm, Column temperature: 30 ℃ ;
Samples: Erbitux® (2 mg/mL), Injection Volume: 10 µL

| Time | %B |
|---|---|
| 0 | 10 |
| 30 | 40 |
| 30.1 | 10 |
| 40 | 10 |



# Erbitux® – *Other Vendor SCX*

Column: Other Vendor SCX PEEK, 5µm, NP 4.0 x 250 mm (P/N: REDACTED);
Mobile phase: CX-1 pH Buffer A; B: CX-1 pH Buffer B;
Flow velocity: 480 cm/h, Detector: UV 280 nm, Column temperature: 30 ℃ ;
Samples: Erbitux® (2 mg/mL), Injection Volume: 10 µL

| Time | %B |
|---|---|
| 0 | 10 |
| 30 | 40 |
| 30.1 | 10 |
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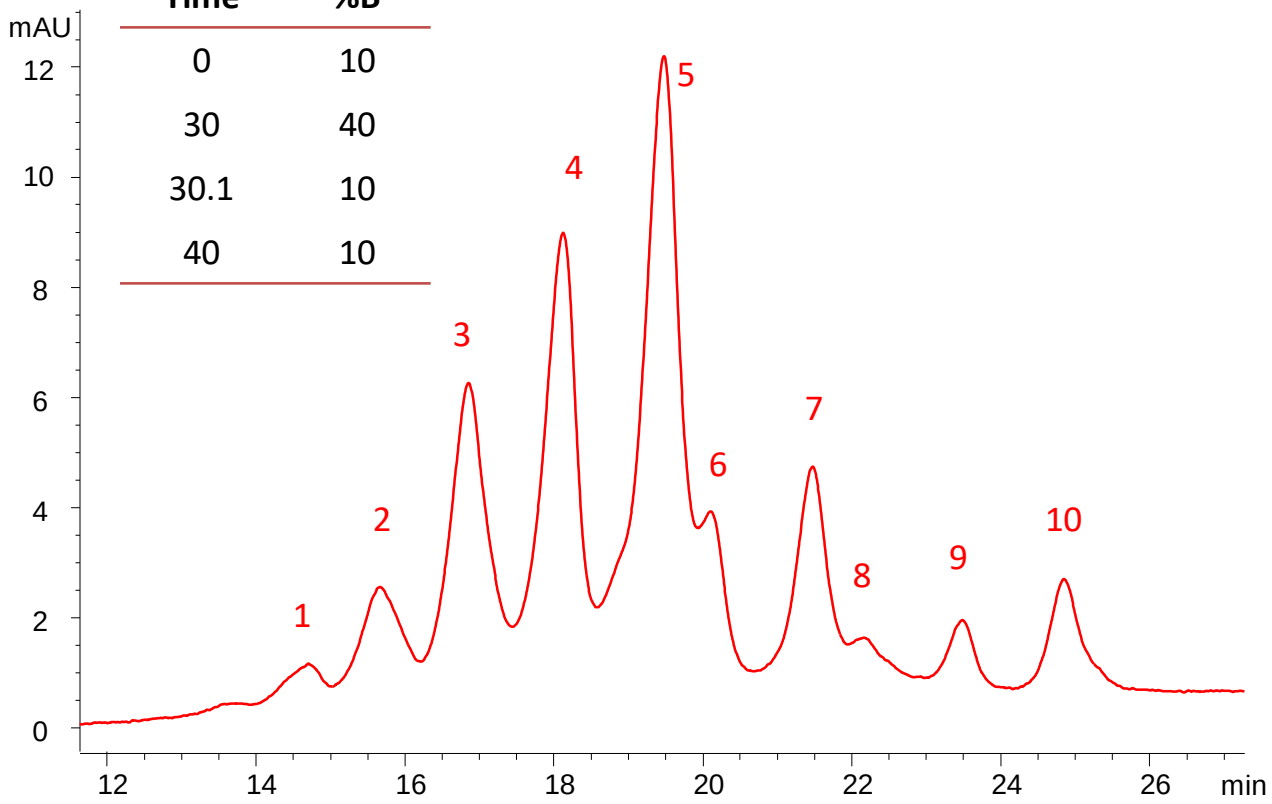

# Erbitux® – Sepax Proteomix SCX vs Other Vendor SCX

PM1024

Columns: Sepax, Proteomix SCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 401NP5P-4625); Other Vendor SCX PEEK, 5µm, NP 4.0 x 250 mm (P/N: REDACTED); Mobile phase: CX-1 pH Buffer A; B: CX-1 pH Buffer B; Flow velocity: 480 cm/h, Detector: UV 280 nm, Column temperature: 30 ℃ ; Samples: Erbitux® (2 mg/mL), Injection Volume: 10 µL

| Time | %B |
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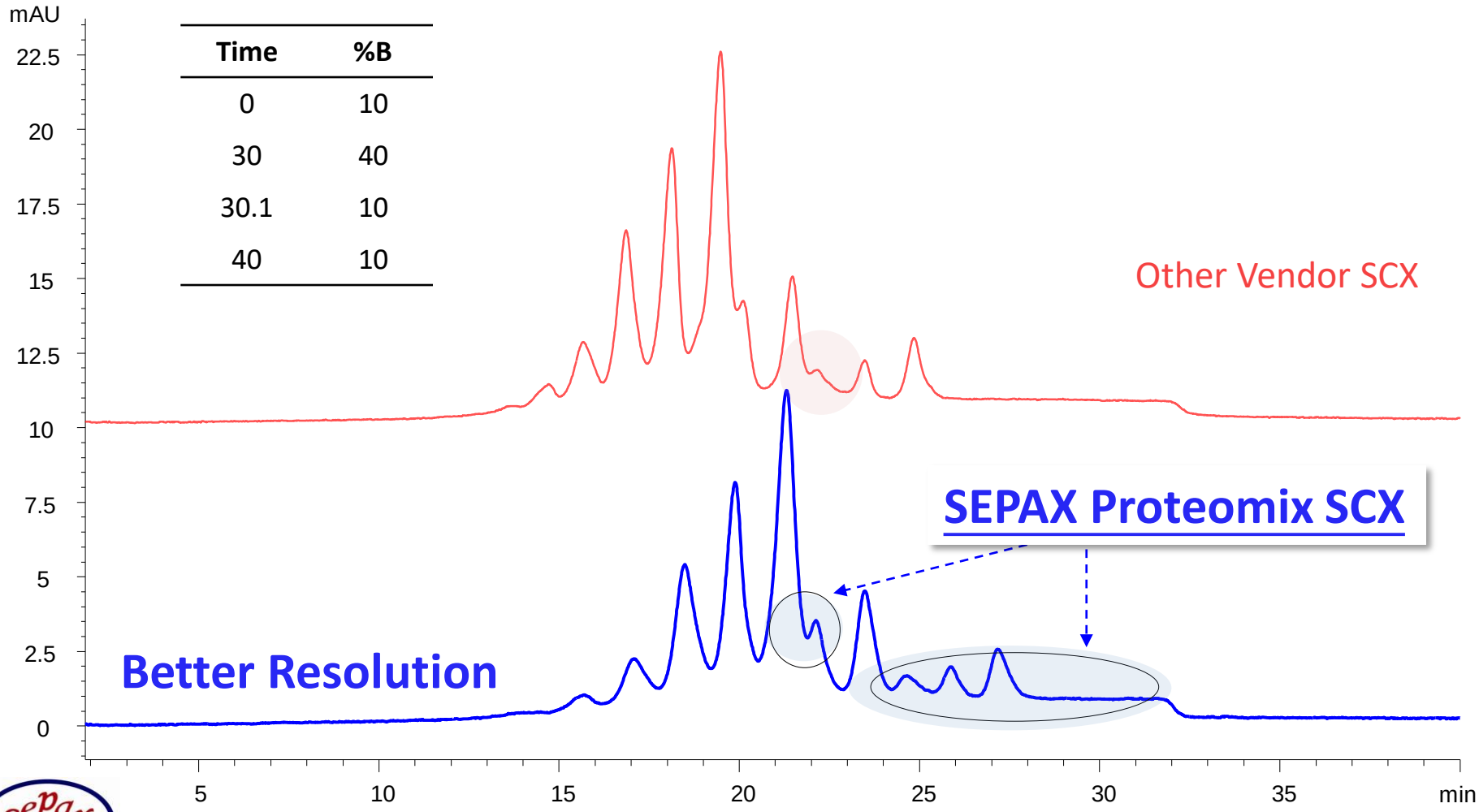

# Erbitux® – Sepax Proteomix SCX vs Other Vendor SCX

PM1024

Columns: Sepax, Proteomix SCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 401NP5P-4625); Other Vendor SCX PEEK, 5µm, NP 4.0 x 250 mm (P/N: REDACTED); CX-1 pH Buffer A; B: CX-1 pH Buffer B; Flow velocity: 480 cm/h, Detector: UV 280 nm, Column temperature: 30 ℃; Samples: Erbitux® (2 mg/mL), Injection Volume: 10 µL

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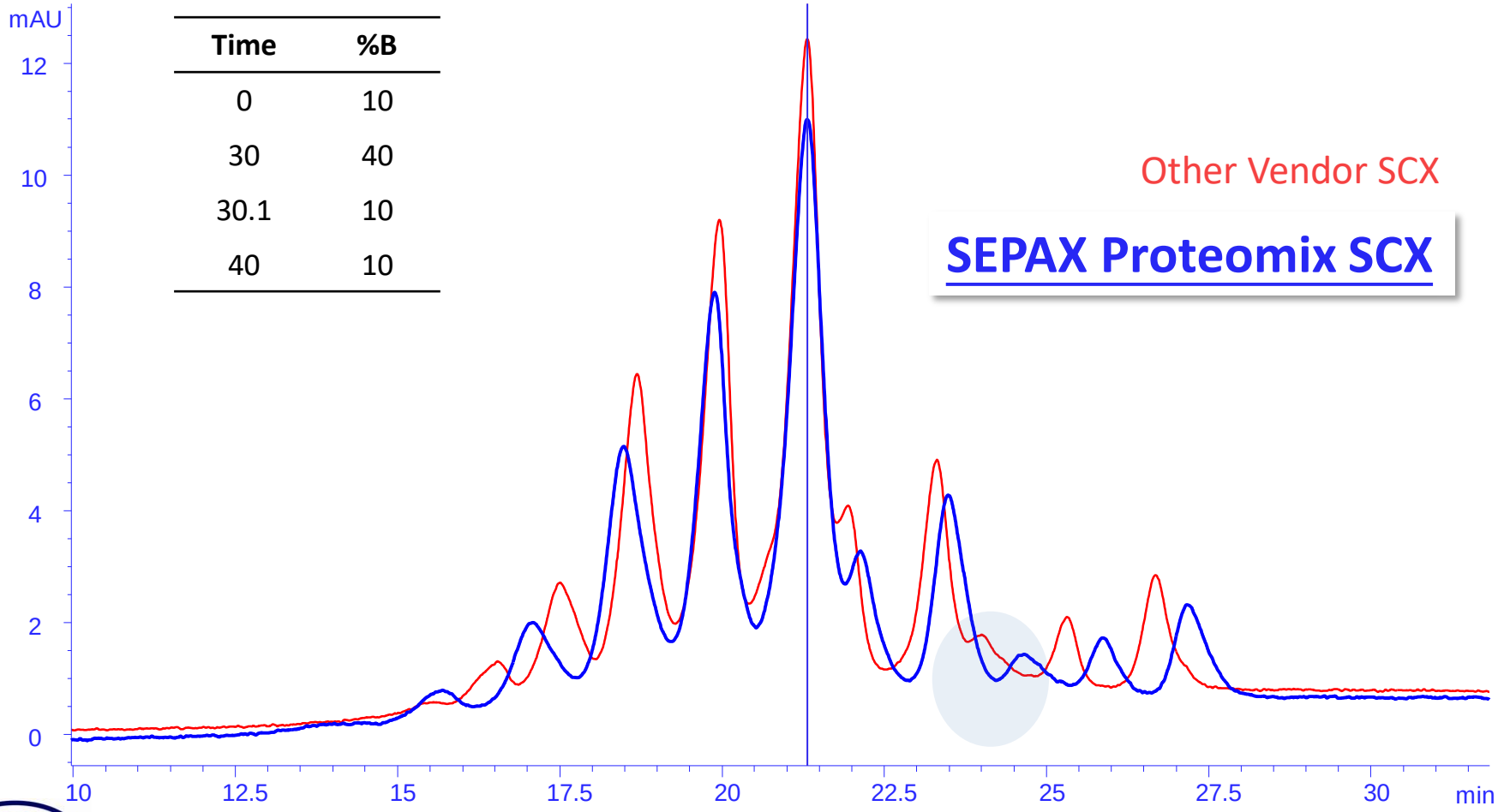
| 40 | 10 |



# Weak Cation Exchange (WCX) Column Comparison

**HPLC Columns**:

Sepax, Proteomix WCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 402NP5P-4625)

Other Vendor WCX PEEK, 10µm, NP 4.0 x 250 mm (P/N: *REDACTED*)

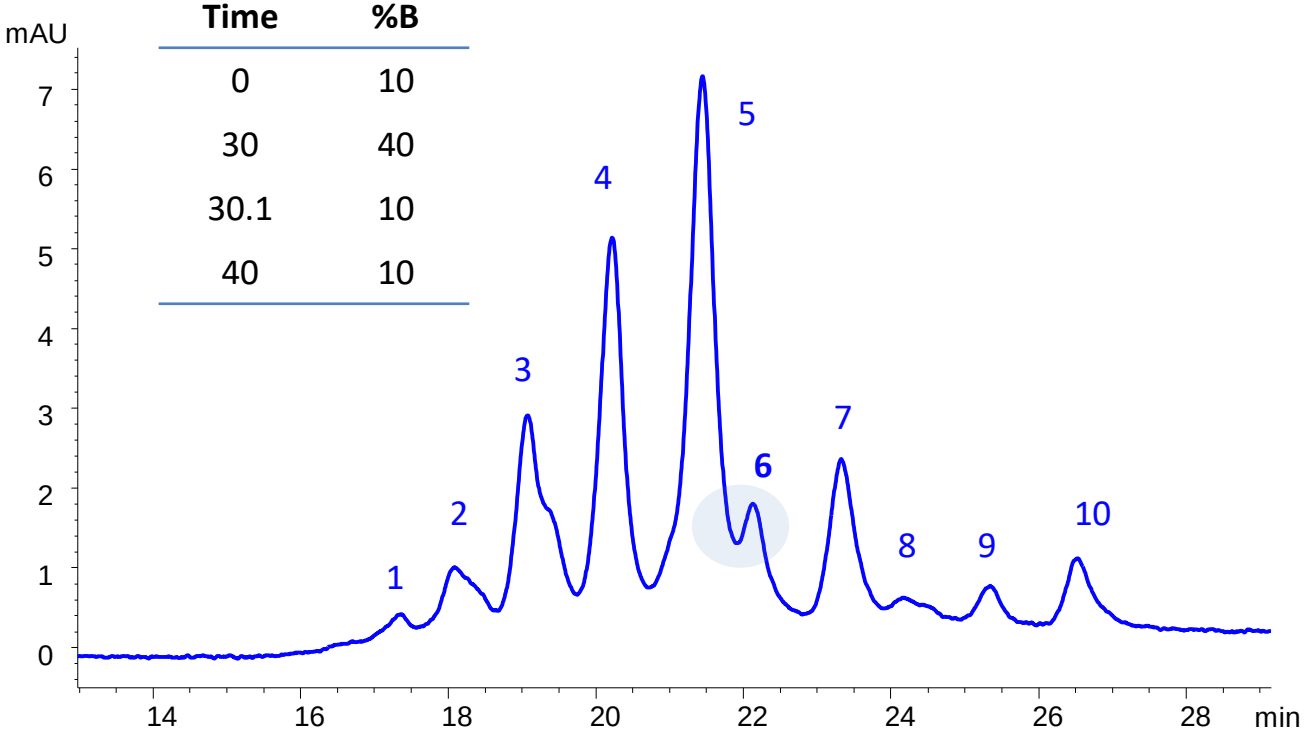
**Flow rates and injection amounts were linearized based on column specifications*

# Erbitux® – ***Sepax*** Proteomix WCX

Column: Sepax, Proteomix WCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 402NP5P-4625);
Mobile phase: CX-1 pH Buffer A; B: CX-1 pH Buffer B; Flow velocity: 480 cm/h,
Detector: UV 280 nm, Column temperature: 30 ℃ ;
Samples: Erbitux® (2 mg/mL), Injection Volume: 5 µL

| Time | %B |
|---|---|
| 0 | 10 |
| 30 | 40 |
| 30.1 | 10 |
| 40 | 10 |

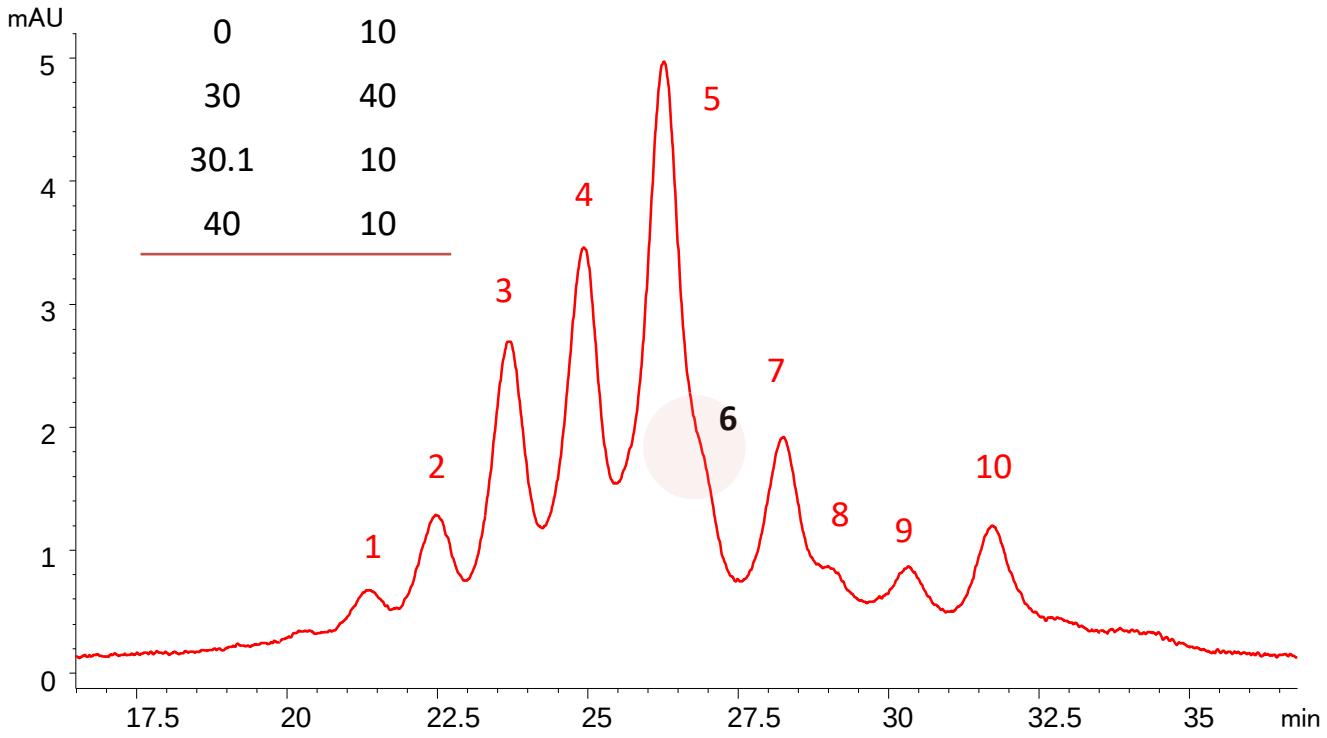



***Sepax*** Proteomix WCX

# Erbitux® – *Other Vendor WCX*

Column: Other Vendor WCX PEEK, 10µm, NP 4.0 x 250 mm (P/N: REDACTED);
Mobile phase: CX-1 pH Buffer A; B: CX-1 pH Buffer B; Flow velocity: 480 cm/h,
Detector: UV 280 nm, Column temperature: 30 ℃ ;
Samples: Erbitux® (2 mg/mL), Injection Volume: 5 µL

| Time | %B |
|---|---|
| 0 | 10 |
| 30 | 40 |
| 30.1 | 10 |
| 40 | 10 |




*Other Vendor WCX*

# Erbitux® – Sepax Proteomix WCX vs Other Vendor WCX

PM1024

Columns: Sepax, Proteomix WCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 402NP5P-4625); Other Vendor WCX PEEK, 10µm, NP 4.0 x 250 mm (P/N: REDACTED); Mobile phase: CX-1 pH Buffer A; B: CX-1 pH Buffer B; Flow velocity: 480 cm/h, Detector: UV 280 nm, Column temperature: 30 ℃; Samples: Erbitux® (2 mg/mL), Injection Volume: 5 µL

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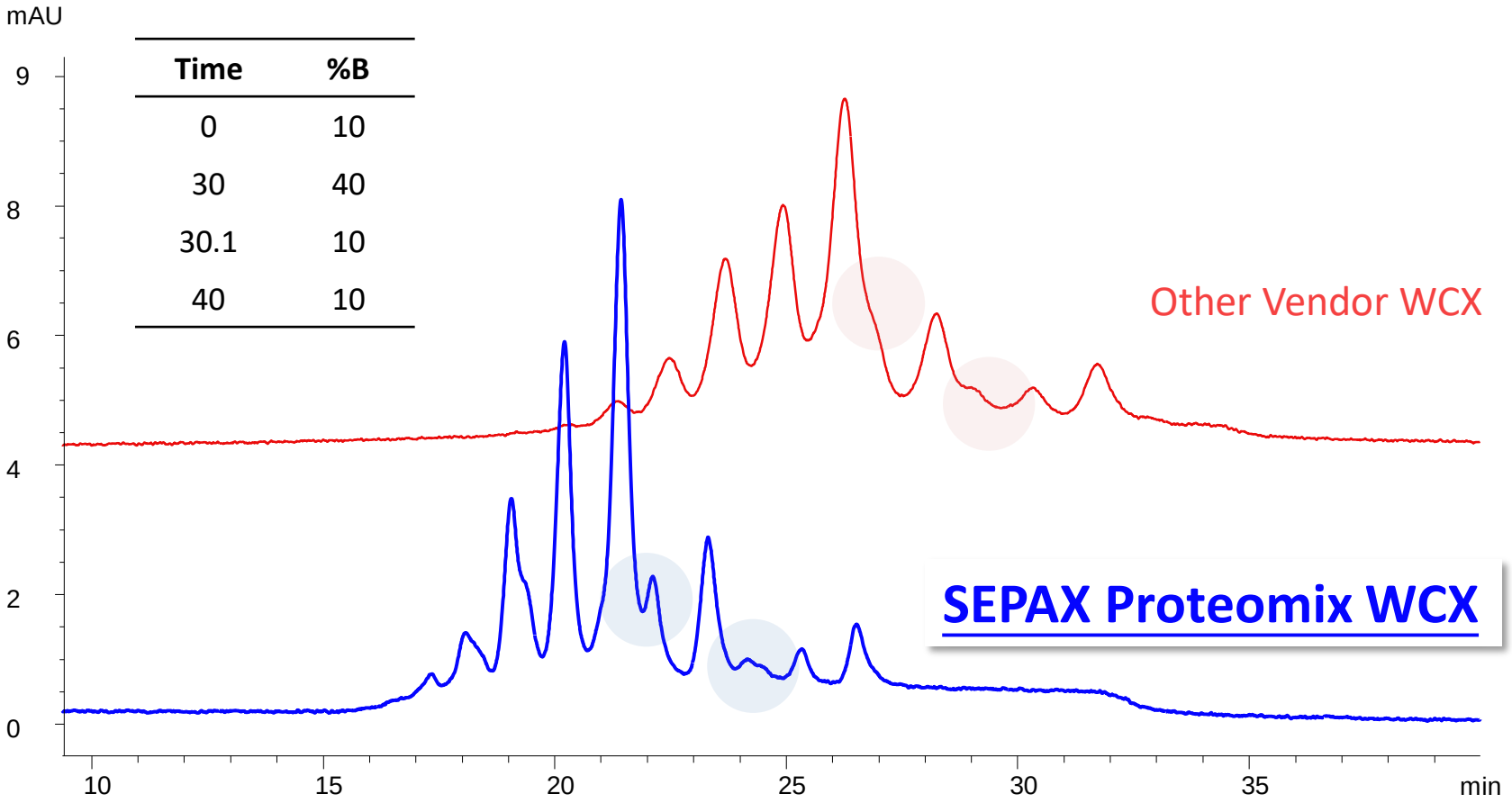

# Erbitux® – Sepax Proteomix WCX vs Other Vendor WCX

PM1024

Columns: Sepax, Proteomix WCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 402NP5P-4625); Other Vendor WCX PEEK, 10µm, NP 4.0 x 250 mm (P/N: REDACTED); Mobile phase: CX-1 pH Buffer A; B: CX-1 pH Buffer B; Flow velocity: 480 cm/h, Detector: UV 280 nm, Column temperature: 30 ℃ ; Samples: Erbitux® (2 mg/mL), Injection Volume: 5 µL

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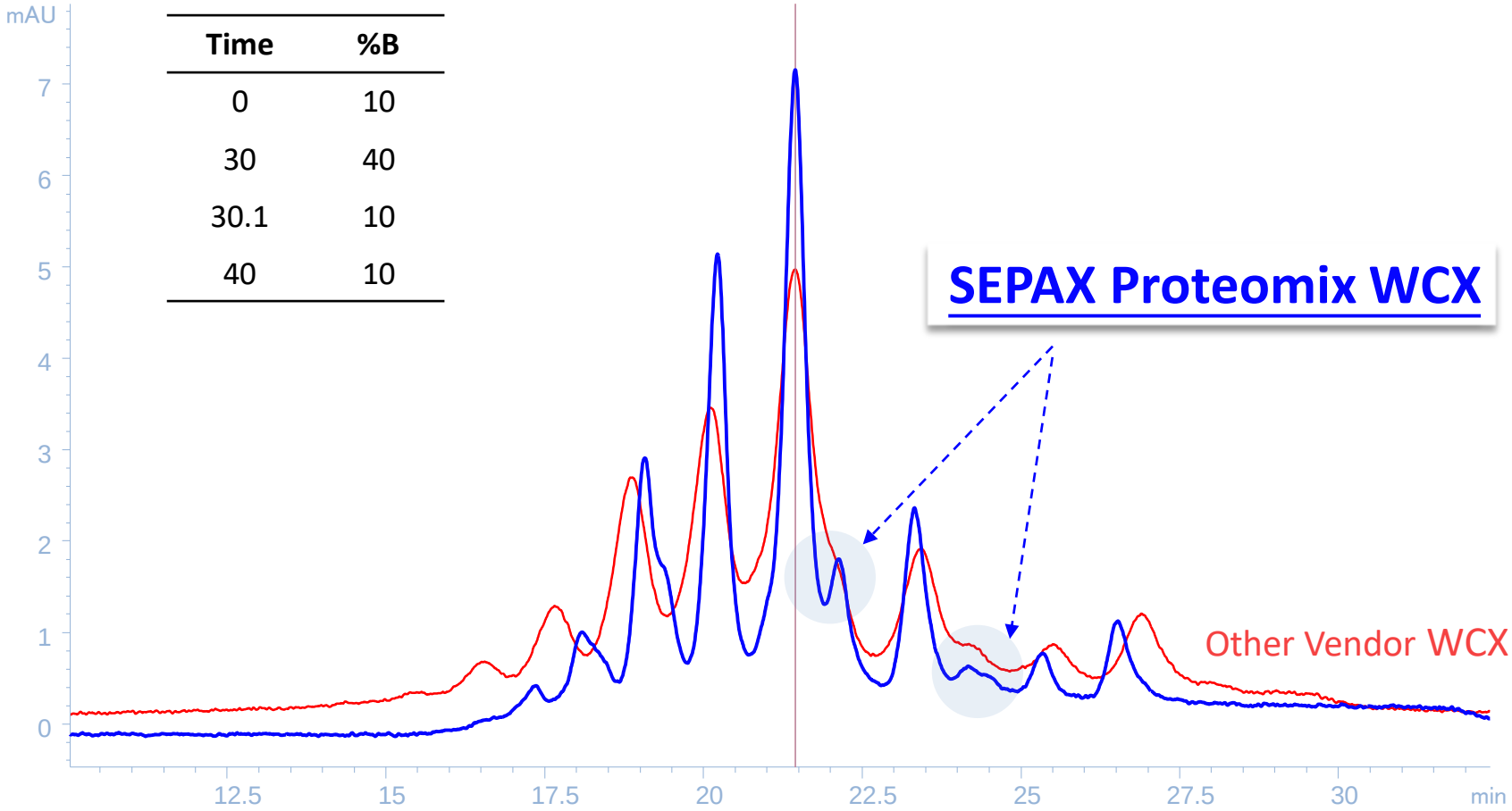
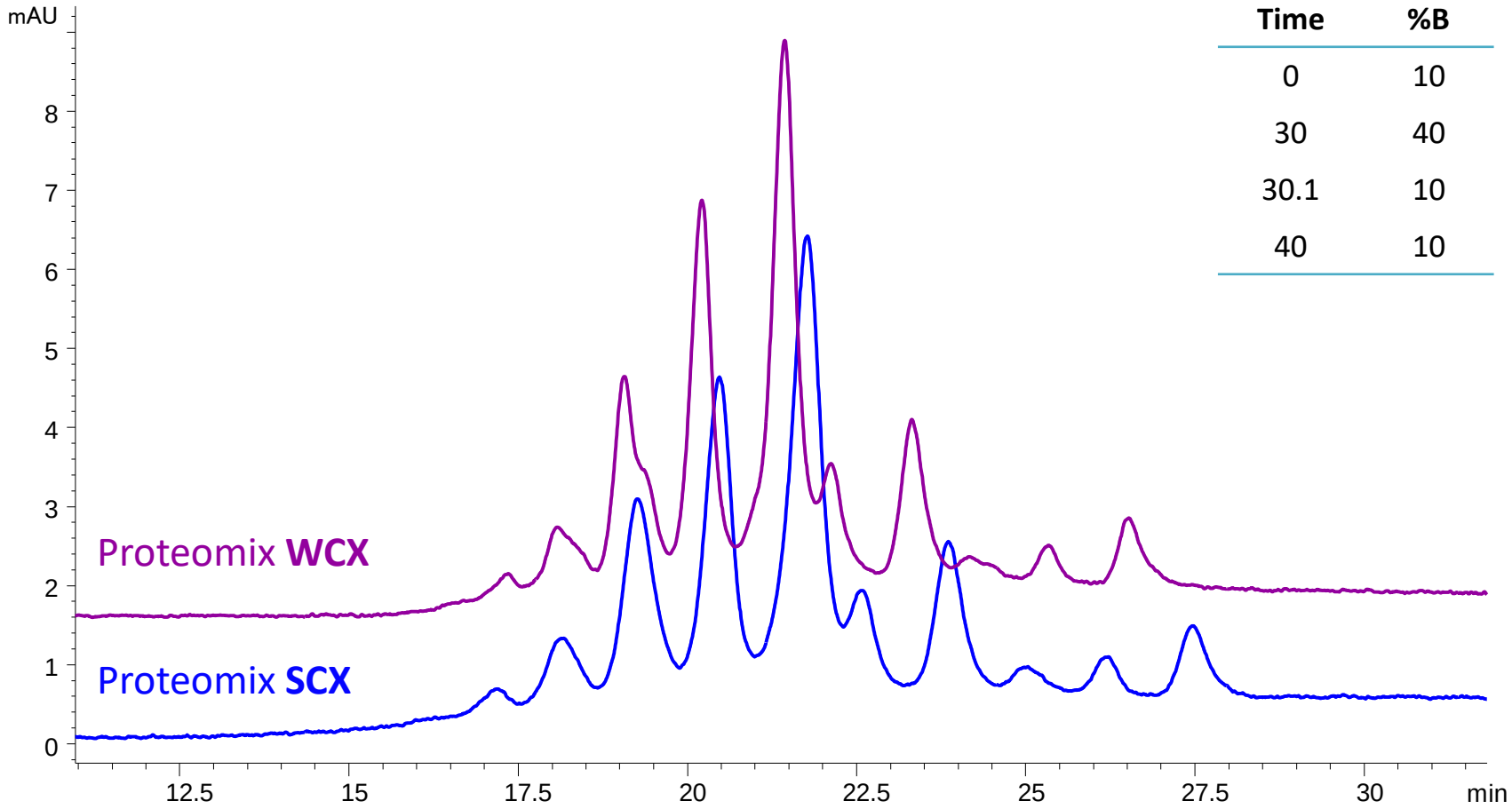


# Erbitux® - SCX vs WCX

PM1024

Columns: Sepax, Proteomix SCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 401NP5P-4625); Sepax, Proteomix WCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 402NP5P-4625); Mobile phase: CX-1 pH Buffer A; B: CX-1 pH Buffer B; Flow velocity: 480 cm/h, Detector: UV 280 nm, Column temperature: 30 ℃ ; Samples: Erbitux® (2 mg/mL), Injection Volume: 5 µL




| Time | %B |
|---|---|
| 0 | 10 |
| 30 | 40 |
| 30.1 | 10 |
| 40 | 10 |

# Erbitux® - *Proteomix* **SCX** vs *Proteomix* **WCX**

PM1024

Columns: Sepax, Proteomix SCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 401NP5P-4625); Sepax, Proteomix WCX-NP5 PEEK, 5µm, NP 4.6 x 250 mm (P/N: 402NP5P-4625); Mobile phase: CX-1 pH Buffer A; B: CX-1 pH Buffer B; Flow velocity: 480 cm/h, Detector: UV 280 nm, Column temperature: 30 ℃ ; Samples: Erbitux® (2 mg/mL), Injection Volume: 5 µL

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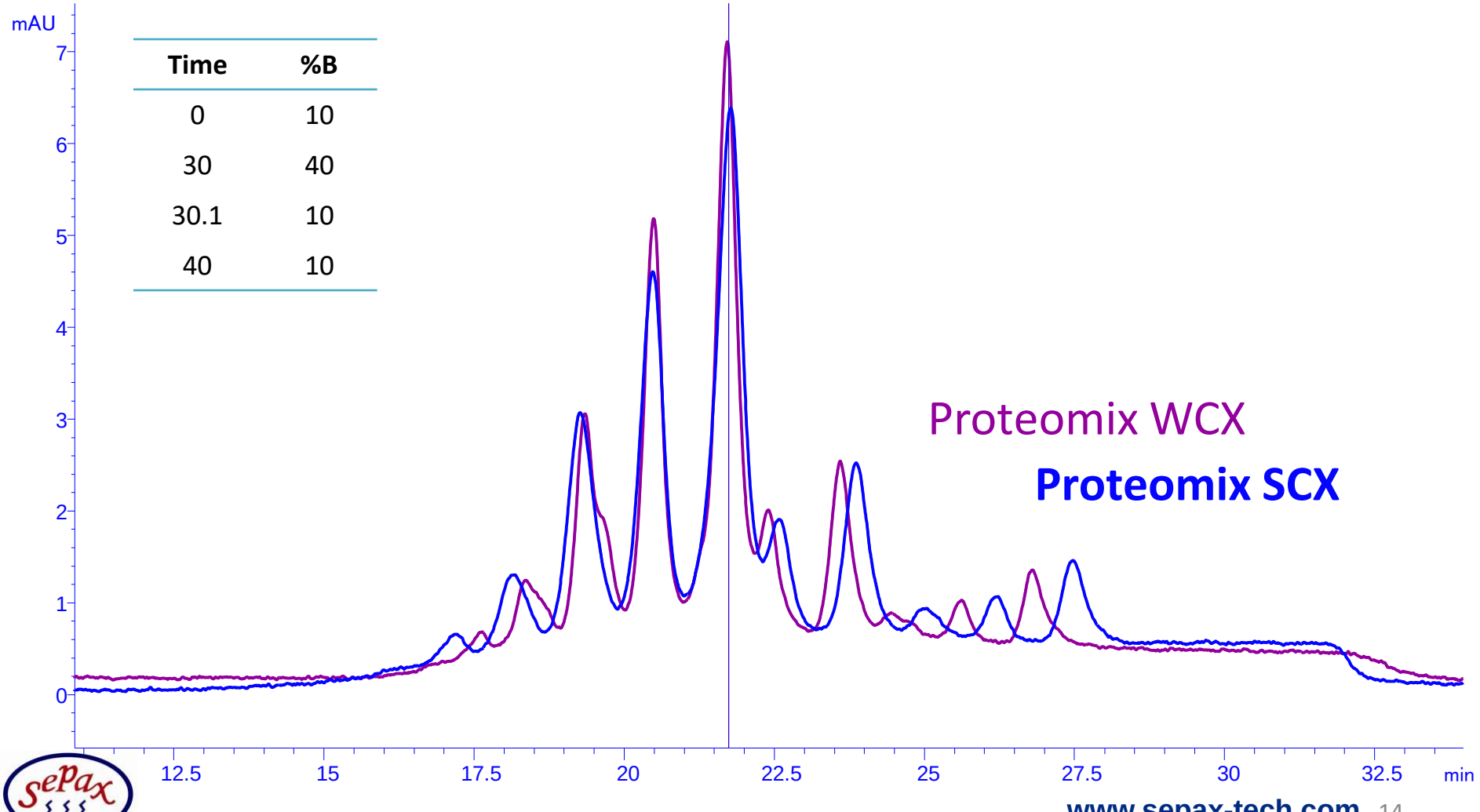

# Additional Information

- **ERBITUX®** (cetuximab) is a commercially available epidermal growth factor receptor (EGFR) antagonist. It is a chimeric monoclonal antibody (mAb) indicated in the treatment of EGFR-expressing cancerous tumors. The approximate molecular weight is 152 kDa and it is composed of the Fv regions of a murine anti-EGFP antibody with constant human IgG1 heavy and kappa light chain regions.

- Structural modifications (variants) of mAbs can be correlated to biological activity and side-effects of a given drug, hence microheterogeneity is closely monitored and studied during all aspects of development and production to ensure product quality is constant.

- Ion Exchange Chromatography (IEX) with elution via pH gradient provides a useful tool for analytical characterization and high resolution separation of surface charge characteristics of antibody variants.

# Isoelectric Points for 25 mAbs (PI)

| | Calculated (Vector NTI) | Calculated (MassLynx) | Measured iCIEF, n = 3 |
|---|---|---|---|
| Adalimumab | 8.7 | 8.9 | 8.9 |
| Atezolizumab. | 8.6 | 8.8 | 8.6 |
| Belimumab | NA | NA | 8.6 |
| Bevacizumab | 8.5 | 8.7 | 8.3 |
| Cetuximab | 8.7 | 8.9 | 8.8 |
| Dalotuzumab | 9.0 | 9.1 | 9.0 |
| Denosumab | 8.8 | 9.0 | 8.9 |
| Eculizumab | 6.0 | 6.4 | 6.1 |
| Elotuzumab | 8.3 | 8.0 | 8.0 |
| Infliximab | 7.1 | 7.4 | 7.6 |
| Ipilimumab | 8.9 | 9.1 | 9.2 |
| Ixekizumab | NA | NA | 8.1 |
| Natalizumab | 8.0 | 7.8 | 7.8 |
| NISTmab | 8.8 | 9.0 | 9.2 |
| Nivolumab | 8.0 | 8.3 | 8.0 |
| Obinutuzumab | 8.7 | 8.8 | 8.6 |
| Ofatumumab | 8.8 | 9.0 | 9.0 |
| Palivizumab | 9.0 | 9.3 | 9.3 |
| Panitumumab | 6.8 | 7.1 | 6.8 |
| Pembrolizumab | 7.4 | 7.8 | 7.6 |
| Pertuzumab | 8.7 | 8.9 | 9.0 |
| Ramucirumab | 8.9 | 8.6 | 9.1 |
| Reslizumab | NA | NA | 7.1 |
| Rituximab | 9.1 | 9.3 | 9.4 |
| Trastuzumab | 8.8 | 9.0 | 9.1 |

**Legend**:
Isoelectric-points of 25 mAbs determined by icIEF and their theoretical calculations based on amino acids sequences. NA: not applicable when the light chains and heavy chains sequences were not available.

**SOURCE**:
Goyon, Alexandre, et al. "Determination of Isoelectric Points and Relative Charge Variants of 23 Therapeutic Monoclonal Antibodies." *NeuroImage*, Academic Press, www.sciencedirect.com/science/article/pii/S1570023217313880?via=ihub#tbl0005.

# Related Application Notes:

| | |
|---|---|
| **ZM1004** | BevacizuMAb and Erbitux Antibodies by Zenix SEC-300 |
| **ZM1016** | Erbitux and its Biosimilar Analysis on Zenix SEC-300 |
| **HICM1005** | HIC separation of Erbitux and Rituximab |
| **ZM1005** | SEC Analysis of Antibody Fragments from Papain Digestion |
| **PM1012** | MAb Innovator vs. its Biosimilar on Protoemix SCX |

**View all application notes here:**
https://www.sepax-tech.com/samples_app.php?sample=All

As always, our technical support team is here to help!

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email: **TechSupport@sepax-tech.com**

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***With our services, we understand the importance of your research and are excited to offer:***

- Fast turn-around (depending on the complexity of the method);
- Full method details and transfer provided;
- Method designed for scalability to preparative chromatography;
- Confidentiality assurance (CDA/NDA)

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## *About SEPAX:*

Sepax Technologies, Inc. based out of Newark, DE, USA has developed a wide range of patented and proprietary technologies in the areas of resin synthesis, surface chemistry and column packing.

Sepax has developed technologies for packing a variety of LC columns, including micro with ID from 75 µm to 1.0 mm, analytical with ID from 2.1 to 7.8 mm, semi-prep and preparative with ID of 10, 21.2, and 30, and DAC/SAC columns with ID from 50 to 800 mm.

## *Sepax Ion Exchange Chromatography:*

In analytical PS/DVB technology, Sepax manufactures a particle size selection of 1.7, 3, 5 and 10 µm. The non-porous particles have unique properties of fast mass transfer and minimized lateral diffusion that enables high resolution separation. Integration of non-porous PS/DVB beads and innovative surface chemistries has led to Proteomix® ion-exchangers, Proteomix® HIC and Antibodix™ WCX product lines. Proteomix® SAX, WAX, SCX and WCX offer a complete solution for ion-exchange separation of biological molecules.